SEQUENCE LISTING

- <110> Microbiological Research Authority Raven, Neil
- <120> DEGRADATION AND DETECTION OF TSE INFECTIVITY
- <130> GWS/22516
- <150> GB 0104696.0
- <151> 2001-02-26
- <150> GB 0100420.9
- <151> 2001-01-08
- <160> 4
- <170> PatentIn version 3.1
- <210> 1
- <211> 11
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- <213> synthetic
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<400> 4

Cys Ile Thr Gln Tyr Gln Arg Glu Ser Gln Ala Tyr Tyr Gln Arg Gly $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$

Ala Ser Cys

																	_
aaa Lys	gaa Glu	ttg Leu	aaa Lys 90	aaa Lys	gac Asp	ccg Pro	agc Ser	gtc Val 95	gct Ala	tac Tyr	gtt Val	gaa Glu	gaa Glu 100	gat Asp	Hi.	401	
													tca Ser			449	
													aat Asn			497	
													gat Asp			545	
					Ser								cct Pro			. 593	
Asp	Asn	Asn	5er 170	His	Gly	Thr	His	Val 175	Ala	Gly	Thr	Val	gcg Ala 180	Ala	Leu	641	
Asn	Asn	Ser 185	Ile	Gly	Val	Leu	Gly 190	Val	Ala	Pro	Ser	Ala 195	tca Ser	Leu	Tyr	689	
Ala	Val 200	Lys	Val	Leu	Gly	Ala 205	Asp	Gly	Ser	Gly	Gln 210	Tyr	agc Ser	Trp	Ile	737	
11e 215	Asn	Gly	Ile	Glu	Trp 220	Ala	Ile	Ala	Asn	Asn 225	Het	Asp	Val	Ile	Asn 230	785	
Het	Ser	Leu	Gly	Gly 235	Pro	Ser	Gly	Ser	Ala 240	Ala	Leu	Lys	Ala	Ala 245	Val	833	
Asp	Lys	Ala	Val 250	Ala	ser	Gly	Val	Vel 255	Val	Val	Ala	Ala	Al a 260	Gly	Asn	881	
Glu	ĞÎy	Thr 265	Ser	Gly	ser	Ser	Ser 270	Thr	Val	Gly	Tyr	Pro 275	Gly	Lys	tac	929	
Pro	5er 280	Val	Ile	Ala	Val	Gly 285	Ala	Val	Asp	Ser	5er 290	Asn	Gln	Arg	gca Ala	977	
8er 295	Phe	Ser	Ser	Val	Gly 300	Pro	Glu	Leu	Asp	Val 305	Met	Ala	Pro	Gly	yta Val 310	1073	
Ser	Ile	Gln	Ser	Thr 315	. Leu	Pro ·	Gly	Asn	Lys 320	Tyr	Gly	Ala	Tyr	325			
Thr	Ser	Met	Ala 330	Ser	Pro	His	Val	Ala 335	Gly	Ala	Ala	Ala	140	Ile	Leu	1121	
Ser	Lys	His 345	Pro	Asn	Trp	Thr	350	Thr	Gln	Val	Arg	8er 355	Ser	Leu	gaa Glu	1169	
Asn	Thr 360	Thr	Thr	Lys	Leu	Gly 365	Asp	Ser	Leu	Tyr	Tyr 370	Qly	Lys	Gly	Leu	1217	
11e 375	Asn	Val	Gln	Ala	Ala 380	Ala	Gln						ggcc			1265	
ccc	gcc	igt 1	tttt	tatte	st tt	ttct	tect		cate	jttc	aat	cege	tcc	ataa	tcgac	g 1325	

gatg	gctc	cc t	ctga	aaat	t tt	aacg	agaa	acg	gcgg	gtt	gacc	cggc	tc a	gtec	cgtaa	1385
cggc	Caac	tc c	tgaa	acgt	c to	aatc	gccg	ctt	cccg	gtt	tccg	gtca	gc t	caat	gccat	1445
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		-			1		-1-		-	•	n		•	•••	•	
Met 1	Arg	GTÀ	Lys	Lys 5	Val	ırp			.0	rea	Pne	ATG	Leu 1	5 5	₽eu	
Ile	Phe	Thr	Met 20	Ala	Phe	Gly	Ser	Thr 25	Ser	Ser	Ala	Gln	Ala 30	Ala	Gly	
Lys	Ser	Asn 35	Gly	Glu	Lys	Lys	Tyr 40	Ile	Val-	Gly	Phe	Lys 45	Gln	Thr	Met	
Ser	Thr 50	Net	ser	Ala	Ala	Lys 55	Lys	Lys	Asp	Val	60 11 e	Ser	Glu	Lys	Gly	
Gly 65	Lys	Val	Gln	Lys	Gln 70	Phe	Lys	Tyr	Val	Asp 75	Ala	Ala	Ser	Val	Thr 80	
Leu	Asn	Glu	Lys	Ala 85	Val	Lys	Glu	Leu	Lys 90	Lys	Asp	Pro	8er	Val 95	Ala	
Tyr	Val	Glu	Glu 100	Asp	His	Val	Ala	Hi= 105	Ala	Tyr	Ala	Gln	Ser 110	Val	Pro	
Tyr	Gly	Val 115	ser	Gln	Ile	Lys	Ala 120	Pro	Ala	Leu	His	8er 125	Gln	Gly	Tyr	
Thr	Gly 130	Ser	Asn	Val	Lys	Val 135	Ala	Val	Ile	Asp	Ser 140	Gly	Ile	Asp	Ser	
8er 145	His	Pro	Asp	Lou	Lys 150	Val	Ala	8er	Gly	Ala 155	Ser	Met	Val	Pro	Ser 160	
Glu	Thr	Asn	Pro	Phe 165	Gln	Asp	Asn	Asn	Ser 170	His	Gly	Thr	His	Val 175	Ala	
Gly	Thr	Val	Ala 180	Ala	Leu	Asn	Asn	Ser 185	Ile	Gly	Val	Leu	Gly 190	Val	Ala	
Pro	Ser	Ala 195	Ser	Leu	Tyr	Ala	Val 200	Lys	Val	Leu	Gly	Ala 205	Asp	Gly	Ser	
Gly	Gln 210	Tyr	Ser	Trp	Ile	Ile 215	Asn	Gly	Ile	Glu	Trp 220	Ala	Ile	Ala	Asn	
Asn 225	Net	Asp	Val	Ile	Asn 230	Met	Ser	Leu	Gly	Gly 235	Pro	Ser	Gly	Ser	Ala 240	
Ala	Leu	Lys	Ala	Ala 245	Val	Asp	Lys	Ala	Val 250	Àla	Ser	Gly	Val	Val 255	Val	
Val	Ala	Ala	Ala 260	Gly	Asn	Glu	Gly	Thr 265	Ser	Gly	Ser	Ser	Ser 270	Thr	Val	
Gly	Tyr	Pro 275	Gly	Lys	Tyr	Pro	Ser 280	Val	Ile	Ala	Val	Gly 285	Ala	Val	Asp	•
Ser	Ser 290	Asn	Gln	Arg	Ala	5er 295	Phe	Ser	Ser	Val	Gly 300	Pro	Glu	Leu	Asp	
Val 305	Met	Ala	Pro	Gly	Val 310	Ser	Ile	Gln	Ser	Thr 315	Leu	Pro	Gly	Asn	Lys 320	
Tyr	Gly	Ala	Týr	Asn 325	Gly	Thr	Ser	Met	Ala 330	Ser	Pro	His	Val	Ala 335	Gly	
Ala	Ala	Ala	Leu 340	Ile	Leu	Ser	Lys	His 345	Pro	Asn	Trp	Thr	Asn 350	Thr	Gln	

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355 360 365
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Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala Ala Gln 370 375 380

<210> SEQ ID NO / 7 <211> LENGTH: 275

<212> TYPE: PRT

<213> ORGANISM: B. amyloliquefacions

<400> SEQUENCE: / 💤

Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu 1 5 10 15

His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ale Val Ile Asp

Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala . 35 40 45

Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His 50 60

Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly 65 70 75 80

Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu 85 90 95

Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu 100 105 110

Trp Ala Ile Ala Asn Asn Het Asp Val Ile Asn Het Ser Leu Gly Gly 115 120 125

Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala 130 135 140

Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly 145 . 150 . 155 . 160

ser ser ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala 165 170 175

Val Gly Ala Val Asp Ser Ser Asn Gln Arg. Ala Ser Phe Ser Ser Val 180 185 190

Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr 195 200 205

Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Net Ala Ser 210 215 220

Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn 225 230 235 240

Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys 245 250 255

Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala 260 265 270

Ala Ala Gln 275

<210> SEQ ID NO / 8 <211> LENGTH: 275

<212> TYPE: PRT <213> ORGANISM: B. subtilis

<400> SEQUENCE:

Ala Gln Ser Val Pro Tyr Gly Ile Ser Gln Ile Lys Ala Pro Ala Leu 1 5 10 15

His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp 20 25 30 Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala 35 40 45 Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly 65 70 75 80 Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu 85 90 95 Asp Ser Thr Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu 100 105 110 Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly 115 120 125 Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser 130 135 140 Ser Gly Ile Val Val Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly 145 150 155 160 Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala 165 170 175 Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala 180 185 190 Gly Ser Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr 195 200 205 Leu Pro Gly Gly Thr Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Thr 210 215 220 Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Thr 225 230 235 240 Trp Thr Asn Ala Gln Val Arg Asp Arg Leu Glu Ser Thr Ala Thr Tyr 245 250 255 Leu Gly Asn Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala 260 265 270 Ala Ala Gln 275

<210> SEQ ID NO / <211> LENGTH: 274

<212> TYPE: PRT
<213> ORGANISM: B. licheniformis

<400> SEQUENCE: #

Ala Gln Thr Val Pro Tyr Gly Ile Pro Leu Ile Lys Ala Asp Lys Val

Gin Ala Gin Gly Phe Lys Gly Ala Asn Val Lys Val Ala Val Leu Asp 20 25 30

Thr Gly Ile Gln Ala Ser His Pro Asp Leu Asn Val Val Gly Gly Ala 35 40 45

Ser Phe Val Ala Gly Glu Ala Tyr Asn Thr Asp Gly Asn Gly His Gly 50

Thr His Val Ala Gly Thr Val Ala Ala Leu Asp Asn Thr Thr Gly Val 65 70 75 80

Ser Ser Gly Ser Gly Ser Tyr Ser Gly Ile Val Ser ly Ile Glu Trp

100 105 110

Ala Thr Thr Asn Gly Met Asp Val Ile Asn Met Ser Leu Gly Gly Ala 115

Ser Gly Ser Thr Ala Met Lys Gln Ala Val Asp Asn Ala Tyr Ala Arg 130

Gly Val Val Val Val Ala Ala Ala Gly Asn Ser Gly Asn Ser Gly Ser 160

Thr Asn Thr Ile Gly Tyr Pro Ala Lys Tyr Asp Ser Val Ile Ala Val 175

Gly Ala Val Asp Ser Asn Ser Asn Arg Ala Ser Phe Ser Ser Val Gly 180

Ala Glu Leu Glu Val Met Ala Pro Gly Ala Gly Val Tyr Ser Thr Tyr 195

Pro Thr Asn Thr Tyr Ala Thr Leu Asn Gly Thr Ser Met Ala Ser Pro 210

His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Leu 225

Gly Ser Ser Phe Tyr Tyr Gly Lya Gly Leu Ile Asn Val Glu Ala Ala Ala Ala Gln

Ala Gln

<210> SEQ ID NO / (-) <211> LENGTH: 269

<212> TYPE: PRT <213> ORGANISM: B. lentus

<400> SEQUENCE: / 10

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro ly Ser Thr Tyr

Ale Ser Leu Asn Gly Thr Ser Het Ale Thr Pro His Val Ale Gly Ale 210

Ale Ale Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile 225

Arg Asn His Leu Lys Asn Thr Ale Thr Ser Leu Gly Ser Thr Asn Leu 245

Tyr Gly Ser Gly Leu Val Asn Ale Glu Ale Ale Thr Arg 260

SEQUENCE CHARACTERISTICS: Sequence variant MC3 of subtilisin from Bacillus subtilis with amino acid mutations N76D, Q103A and Y104I (shown in bold type)

SEQ ID NO 54 | LENGTH: 275

TYPE: PRT

ORGANISM: B. subtilis

SEQUENCE: 6

Ala Gln Ser Val Pro Tyr Gly Ile Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp 25 Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asp Asn Ser Ile Gly Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu 90 Asp Ser Thr Gly Ser Gly Ala Ile Ser Trp Ile Ile Asn Gly Ile Glu 105 1.00 Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly 120 Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser 140 135 Ser Gly Ile Val Val Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly 150 155 Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala 170 165 Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala 180 185

SEQUENCE CHARACTERISTICS: Sequence variant of subtilisin from Bacillus lentus with amino acid mutations N74D, S101A and V102I (shown in bold type)

SEQ ID NO 26 12 LENGTH: 269 TYPE: PRT

ORGANISM: B. lentus

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp 20 25 Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser .40 Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr 55 His Val Ala Gly Thr Ile Ala Ala Leu Asp Asn Ser Ile Gly Val Leu 70 75 Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala 85 . 90 Ser Gly Ser Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala 100 105 Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser 120 125 Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly 135 140 Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser 155 Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln 165 170 Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile 180 185 Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr 200 205 Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala 215 220 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile 230 235 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu 245 250 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg 260 265